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The Role of Plakoglobin in Breast Cancer Cell Motility and Invasion

Introduction The goal of this project was to determine how plakoglobin (PG) affects cell motility. Others in the Brackenbury laboratory previously isolated variants of the PAM212 keratinocyte cell line that expressed low levels of plakoglobin, did not form compact colonies, and had lost contact suppression of motility. These findings implied that plakoglobin is a significant regulator of cell movement. I proposed to analyze how plakoglobin exerted its effect, to determine whether plakoglobin acted in a structural capacity, such as a docking protein or signal transducer, or whether it acted as a transcriptional activator, possibly controlling expression of genes required to suppress motility. During the first year of this fellowship, I found that the original PAM212 cell model was unsuitable for further investigation and developed a new model system for analysis, in the process verifying that plakoglobin is required for contact regulation of movement. I also characterized a portion of the human plakoglobin gene (Warren, 2002), correcting a significant error in the literature and produced mutant plakoglobin constructs that will soon be used to analyze how plakoglobin suppresses movement.

Detailed Report Following submission of my proposal, I began experiments with the parental and variant PAM212 mouse keratinocyte cell lines. I quickly found, however, that, although previously stable for more than two years, these lines had reverted, becoming less motile and expressing levels of plakoglobin similar to the normal, parental cell line. Despite several months' effort, all attempts to recover the original lines, or to determine the source of the instability, were unsuccessful.

To develop an alternative experimental system, we explored the possibility of isolating keratinocytes from PG $-/-$ mice, produced by Dr. Rolf Kemler. Dr. Kemler informed us that Dr. Eliane Mueller had already developed such lines and had a publication in press describing their initial characterization (Caldelari, 2001). We began collaborating with Dr. Muller and obtained the PG $-/-$ and control PG $+/ -$ keratinocytes cells from her. These cells have been used for the experiments described in the remainder of this report.

The phenotype of the PG $-/-$ keratinocyte cells differed somewhat from that of the PG-deficient PAM212 cells. The PG $-/-$ keratinocyte cells formed stable adhesions with each other and often collected into compact colonies. It was striking, however, that the PG $-/-$ keratinocytes

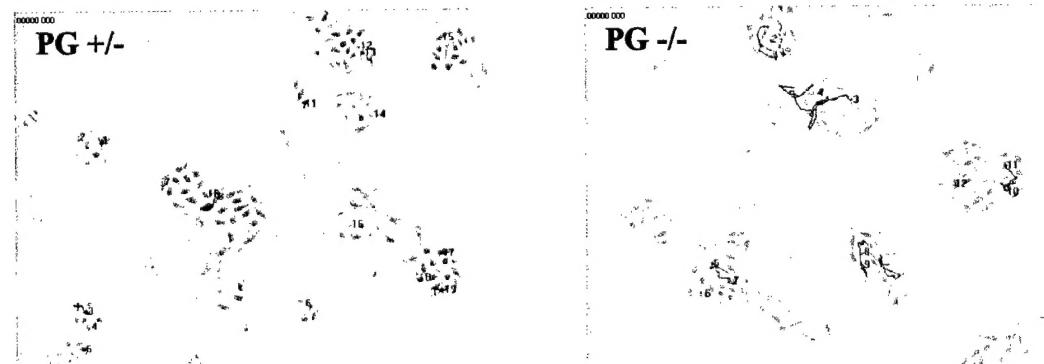


Figure 1. PG $-/-$ Cells Move to a Greater Extent than PG $+/ -$ Cells. Mouse keratinocytes were plated on glass coverslips and incubated in normal medium for 24 hours. Medium was then replaced with supplement-free medium except for antibiotic, and cells were incubated for 24 hours. Time-lapse video microscopy was then used to assay cell movement for 60 frames, one frame taken every 15 min.

remained motile within these colonies, like the PG-deficient PAM212 cells. This led me to analyze the cells in a different way. Instead of timing the duration of new contacts, I analyzed the total distance traveled of individual cells, all within colonies. By doing this, the cells in colonies could be compared, thereby bypassing the effects of cell-cell contact. The criteria for selecting cells to track were (1) the cells were initially in colonies and (2) the cells remained in focus for the duration of the assay. Sample tracings of cells are shown in Figure 1. As shown, most PG +/- cells move very little, whereas PG -/- cells migrate to a much greater degree. The quantification of these data is shown in Table 1. As a control, I counted how fast single cells not in contact with other cells migrated, and the rate of movement was similar for both PG +/- and PG -/-. In sum, PG null cells in colonies moved a total distance three times that of the control cells.

Table 1. Total Distance of Cell Migration for PG +/- and PG -/- Keratinocytes

	Total Distance/15 hr	Average Distance/15 min.
PG +/-	115 ± 71	1.9 ± 1.2
PG -/-	353 ± 140	6 ± 2.4

The significant difference in motility between the PG null and control keratinocytes establishes a reproducible and robust method for future proposed assays. These assays include infecting the PG null cells PG mutant constructs to test the hypothesis that plakoglobin controls cell movement via a transcription mechanism. The constructs I have made or acquired are depicted in Figure 2. These mutant PG genes have been transferred to an adenovirus vector and virus stocks are currently being produced.

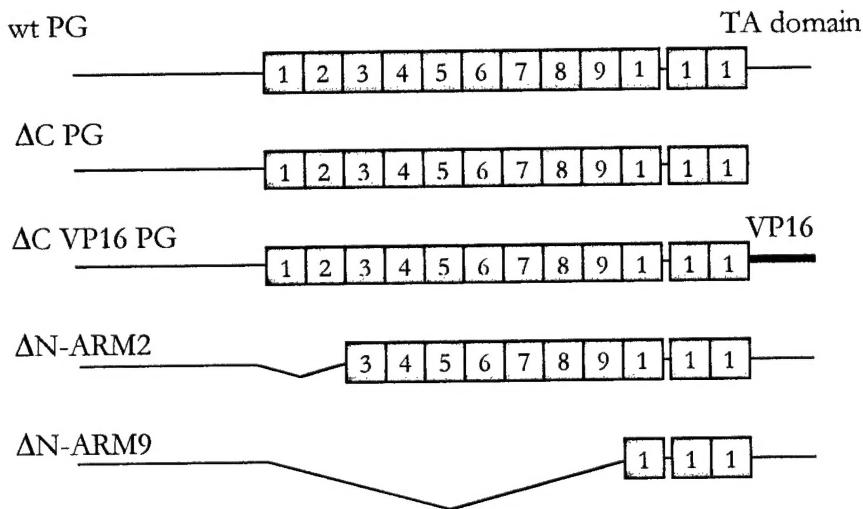


Figure 2. Wild-type PG and Mutant Constructs. C-terminal deletion and mutation constructs were created for this project. N-terminal deletion constructs were acquired from Dr Pamela Cowin.

To test the role of plakoglobin in regulating motility of mammary epithelial cells, I obtained several human breast epithelial cell lines for characterization. The goal was to identify a line with appropriate regulation of motility, then develop a variant with diminished plakoglobin

expression either by repeated light trypsinization, as was done for the mouse keratinocyte line, or genetically by insertion /deletion. I first characterized the movement of the human breast epithelial cell line MCF10A. Video time-lapse microscopy revealed that these cells did not remain in compact colonies for the duration of the assay, and were thus deemed unsuitable. I then tested more "normal" human breast epithelial cell lines, which also proved unsuitable for analysis for different reasons. A cell line from Mina Bissell had may have been suitable since the colonies seemed somewhat stable, but I found, by FISH, that it contained three copies of the plakoglobin gene, thus severely hindering any attempts at deleting the gene from the chromosome. As yet, therefore, no highly suitable mammary epithelial line has been identified.

Due to personal circumstances, I have recently moved to another city and will no longer be working on this project. I have contacted appropriate personnel at the army to inform them of my move and my wish to leave this grant with my advisor to continue the project.

References

Caldelari, R., et al. (2001) "A Central Role for the Armadillo Protein Plakoglobin in the Autoimmune Disease Pemphigus Vulgaris." J. Cell Biol. **153**(4): 823-834.

Warren, M. A., A. Koshoffer, et al. (2002). "Structure of the 5' Portion of the Human Plakoglobin Gene." J Invest Dermatol **119**(1): 196-197.

Key Accomplishments

- Correct a published error in the genomic sequence of plakoglobin (Warren, 2002)
- Verify that plakoglobin is required for contact regulation of cell movement
- Create mutant constructs of plakoglobin to test cell motility

Reportable Outcomes

Warren, M. A., A. Koshoffer, et al. (2002). "Structure of the 5' Portion of the Human Plakoglobin Gene." J Invest Dermatol **119**(1): 196-197.